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Case Report

CFTR mutation in an Arab patient: Clinical and functional features of 875+1G→A/875+1G→A genotype

Elide Spinelli [a], Manuela Seia [b], Paola Melotti [c], Eleonora Marchina [a], Rita Padoan [d,*]

[a] *Biology and Genetics Unit, Department of Biomedical Sciences and Biotechnologies, University of Brescia, Italy*
[b] *Molecular Genetics Laboratory, IRCCS Fondazione Ospedale Maggiore Policlinico, Mangiagalli, Regina Elena, Milano, Italy*
[c] *Cystic Fibrosis Regional Centre, Ospedale Maggiore Borgo Trento, Verona, Italy*
[d] *Cystic Fibrosis Regional Support Centre, Department of Paediatrics, AO Spedali Civili, Brescia, Italy*

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Abstract

Cystic fibrosis (CF; MIM# 219700) is the most frequent recessive disease in Caucasian patients. However, immigration from the Middle East and Africa to Europe is revealing different CFTR mutations.

Here, we have described an 875+1G→A mutation, found for the first time in a homozygous state in an 8 yr old boy. He was the child of a couple of Egyptian first level cousins, both carriers of the mutation.

The functional test revealed the 875+1G→A to be a severe mutation, leading to defective protein function as detected by nasal potential difference (NPD) measurements.

Keywords: Arab mutations; CFTR; Cystic fibrosis; 875+1G→A

1. Introduction

Cystic fibrosis (CF; MIM# 219700) is the most common recessive autosomal Caucasian disorder, carriers being 1:25 and with an incidence of 1:2500–1:4400 in different populations decreasing from north-west to south-eastern Europe [1,2].

First cloned in 1989, cystic fibrosis transmembrane conductance regulator (CFTR) gene (or ABCC7) (MIM 602421), located on chromosome 7q31.2 encodes for a transmembrane ATP-binding cassette protein, which principally functions as an ion channel, regulating chloride ion traffic at the apical membrane of epithelial cells [3].

More than 1600 CFTR mutations are currently reported and listed in the CFTR mutation database [4]. Recently, in our country an increasing number of newborns from immigrant couples from Mediterranean populations undergo neonatal screening programmes for cystic fibrosis, available for 80% of the whole neonatal population. Here, we would like to add to the spectrum of CFTR mutations describing clinical, genetic and functional data of an Egyptian CF patient with a rare homozygous genotype.

2. Case report

GG, male, 8 yrs old, is the last of five siblings, born in Italy in 2000 from a couple of Egyptian first cousins. One sister, reported to be affected by cystic fibrosis, died at 2 months. A first cousin is affected and alive (Fig. 1). Newborn screening was positive, with persistent hypertrypsinogenemia (127 ng/ml; 80 ng/ml at recall), while sweat chloride values in the first months of life were not considered completely diagnostic (sweat chloride: 54.64 and 57.30 mmol/l). First level mutation screening was negative for delF508 and other 31 mutations [5], then the infant was referred to the Regional CF Centre to

* Corresponding author. Cystic Fibrosis Regional Support Centre, Department of Paediatrics, Piazzale Spedali Civili 1, 25123 Brescia, Italy. Tel.: +39 0303996287; fax: +39 0303996028.
E-mail address: ritaf54@gmail.com (R. Padoan).

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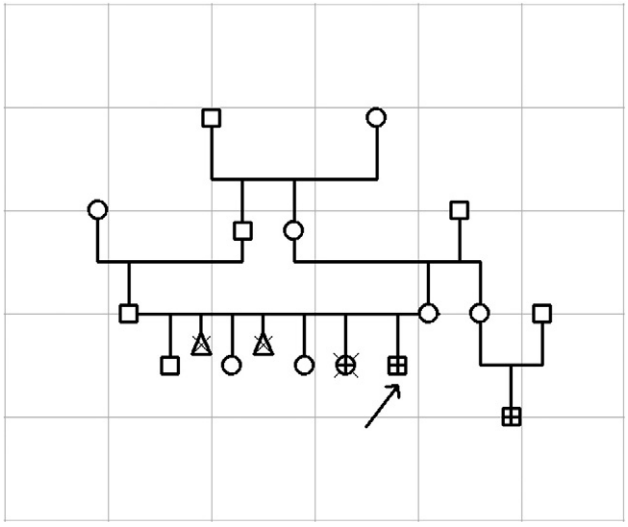

Fig. 1. The patient's family tree.

undergo the follow-up programme for screened infant with borderline sweat test. Due to linguistic and logistic difficulties and to their different cultural backgrounds, the parents underestimated the importance of the diagnostic assessment, so the patient was lost to follow-up.

In April 2004, he was sent to the CF Support Centre at the Paediatric Department of Brescia (third level hospital) from a peripheral hospital. In the first four years of life, he was often hospitalized (more than 2 per year) for wheezing, recurrent bronchopneumonia, chronic cough, and failure to thrive.

A repeated sweat test was performed and CF diagnosis was confirmed (mean NaCl value: 95 mmol/l; cut-off value: 50 mmol/L). Pancreatic status was sufficient (faecal elastase values >500 μg/g; faecal fat assessment <3%).

The child was taken in charge by our CF clinic, daily chest physiotherapy started together with antibiotic therapy when needed, salt and vitamin supplementation. There was no further hospitalization after diagnosis and proper management of the disease was started. Clinical symptoms were limited to upper respiratory tract infections. Latest chest X-ray and CT thoracic scan (2008) showed no pleuro-parenchymal lesions, no bronchiectasies, and no hyperinflation. Ultra sound abdominal examination (2008) showed no hepatic or pancreatic lesion. No nasal polyposis was detected with rhinofibroscopy. No infection by *Staphylococcus aureus* or *Pseudomonas aeruginosa* was ever detected. Growth is on the 75th percentile for height and weight.

3. CFTR sequencing and functional analysis

Extensive molecular analysis was performed using: (i) screening for frequent mutations using different commercial assays for CFTR gene; (ii) scanning of the 27 exons and their boundaries using denaturing high pressure liquid phase chromatography (DHPLC) [6]; (iii) screening for large CFTR rearrangements using a MLPA assay which enabled detection of 20% of the rearrangements of previously unidentified alleles in CF patients [7].

Analysis identified the 875+1G→A mutation on both chromosomes with no other alterations. Parents were tested and found to be both healthy carriers for 875+1G→A.

The mutation leads to a single nucleotide substitution (G→A) in the first nucleotide of intron 6a of CFTR gene. The consequence is an mRNA splicing defect, as described in 1994 by Duarte et al. who first reported the mutation [4]. No homozygote case has ever been described and this is the first report to our knowledge of a homozygous patient.

A functional analysis was performed to assess CFTR protein residual function: the patient was then referred to Verona CF Regional Centre for a nasal potential difference (NPD) test. NPD measurements were performed following the standardized procedure [8] consistent with the recommendations provided by the multicenter basic protocol [9]. In particular, PD was determined by measuring the PD between a Ringers-filled exploring catheter on the nasal mucosa and a reference bridge (21-gauge needle filled with Ringers solution) inserted into the forearm subcutaneous space.

Each nostril was examined using an otoscope; the inferior turbinate was explored for the site of the most negative voltage using a catheter (PE50 tubing, Fisher Scientific BD) connected to an electrode. Baseline PD was perfused with Ringers and subsequently the responses perfused to amiloride (1×10^–4 M), chloride-free, isoproterenol (1×10^–5 M) and ATP (1×10^–4 M) were sequentially acquired for a minimum of 3 min/each obtaining a period of stable signal for at least 30 s. We perfused the solutions at 37 °C to obtain a larger activated chloride conductance [10].

PowerLab (ADInstruments) software was used to acquire and analyse the data. The data acquisition system ML870 PowerLab 8/30 was used in connection with a voltmeter with an impedance of 8 W (Iso Millivolt Meter, World Precision Instruments, Sarasota (WPI), FL, USA).

We measured on the left nostril basal PD of 28.96 mV, on the right 21.55 mV (Fig. 2). The amiloride response was 15.89 mV

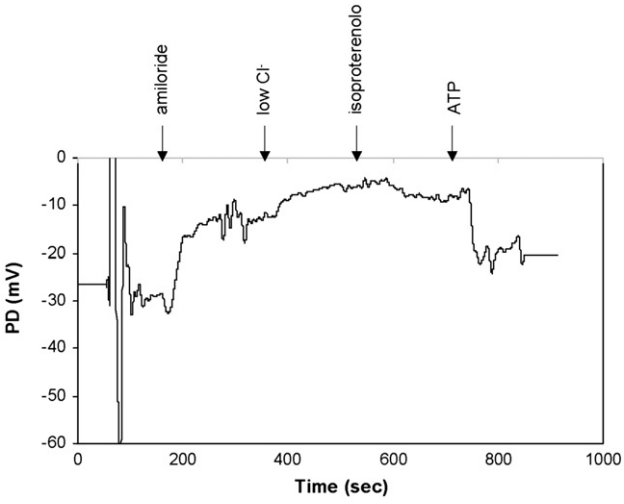

Fig. 2. NPD measurements. The agents indicated at the bottom were added at the times (seconds) shown on the *x* axis. On the *y* axis, PD are expressed in mV. The graph was obtained during measurements of the left nostril and is representative of both nostrils.

and 14.40 mV, chloride-free response 6.89 mV and 2.71 mV, isoproterenol response −2.31 mV and −0.74 mV on the left and right, respectively (Fig. 2). The results of the exponential formula proposed by Wilschanski et al. [11] were 1.33 on the left and 1.15 on the right, and our values were below 0.9 in non-CF subjects and >0.9 in CF patients (sensitivity 93.1%, specificity 94.4%). The results in our patient were therefore consistent with CF. Following ATP exposure hyper-polarization was induced as shown in Fig. 2.

4. Conclusion

Clinical, molecular and CFTR functional data of an Arab CF boy homozygous for the splicing mutation 875+1G→A are shown.

In order to clarify the functional properties of the homozygous genotype and identify the severity of the mutation, we performed a NPD study.

According to the consensus statement for CF diagnosis [12] and the CF diagnostic algorithm [13], NPD may detect the abnormal transport of sodium and chloride characteristic of CF and assist in the diagnosis or exclusion of CF [14,15]. Abnormalities of the CFTR transepithelial function correlate with the number and severity of CFTR gene mutations [16] and we found that amiloride, Cl^- free and isoproterenol responses were all consistently affected with CF diagnosis.

Beyond diagnosis in individual patients, electrophysiological tests could be complementary to highly sensitivity genetic analysis and should be available when rare mutations are detected.

Functional analysis performed on our homozygous patient showed how 875+1G→A mutation deeply affects the CFTR function leading to a very small amount of protein production. 875+1G→A can be defined as a severe mutation, as suspected in the first mutation report. This functional evaluation is very important for rare mutations, in particular in homozygous subjects, as in this case. What remains unclear is why in our patient pancreatic function did not seem to be affected.

Our patient's history underlines the importance to increase the knowledge on CFTR gene alterations and their functional effect: homozygote patients for CFTR mutations must be assessed for *in vivo* functional study to classify as yet uncharacterised CFTR mutations.

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References

[1] Bobadilla JL, Macek Jr M, Fine JP, Farrell PM. Cystic fibrosis: a worldwide analysis of CFTR Mutations-correlation with incidence data and application to screening. Hum Mutat 2002;19:575–606.
[2] Farrell PM. The prevalence of cystic fibrosis in the European Union. J Cyst Fibros 2008;7(5):450–3.
[3] Hodson M, Geddes D, Bush A. Cystic fibrosis. 3rd ed. London: Hodder Arnold; 2007.
[4] http://www.genet.sickkids.on.ca/cftr (05/09/08).
[5] Corbetta C, Seia M, Bassotti A, Ambrosioni A, Giunta A, Padoan R. Screening for cystic fibrosis in newborn infants: results of a pilot programme based on a two tier protocol (IRT/DNA/IRT) in the Italian population. J Med Screen 2002;9:60–3.
[6] Le Maréchal C, Audrézet MP, Quéré I, Raguénès O, Langonné S, Férec C. Complete and rapid scanning of the cystic fibrosis transmembrane conductance regulator (CFTR) gene by denaturing high-performance liquid chromatography (D-HPLC): major implications for genetic counselling. Hum Genet 2001;108(4):290–8.
[7] Paracchini V, Seia M, Coviello D, Porcaro L, Costantino L, Capasso P, et al. Molecular and clinical features associated with CFTR gene rearrangements in Italian population: identification of a new duplication and recurrent deletions. Clin Genet 2008;73(4):346–52.
[8] Ahrens RC, Standaert TA, Launspach J, Han SH, Teresi ME, Aitken ML, et al. Use of nasal potential difference and sweat chloride as outcome measures in multicenter clinical trials in subjects with cystic fibrosis. Pediatr Pulmonol 2002;33(2):142–50.
[9] Schüler D, Sermet-Gaudelus I, Wilschanski M, Ballmann M, Dechaux M, Edelman A, et al. Basic protocol for transepithelial nasal potential difference measurements. J Cyst Fibros 2004;3(2):151–5.
[10] Boyle MP, Diener-West M, Milgram L, Knowles M, Foy C, Zeitlin P, et al. Cystic Fibrosis Therapeutics Development Network Nasal PD Study Group. A multicenter study of the effect of solution temperature on nasal potential difference measurements. Chest 2003;124(2):482–9.
[11] Wilschanski M, Famini H, Strauss-Liviatan N, Rivlin J, Blau H, Bibi H, et al. Nasal potential difference measurements in patients with atypical cystic fibrosis. Eur Respir J 2001;17(6):1208–15.
[12] Rosenstein BJ, Cutting GR. The diagnosis of cystic fibrosis: a consensus statement. Cystic Fibrosis Foundation Consensus Panel. J Pediatr 1998;132 (4):589–95.
[13] De Boeck K, Wilschanski M, Castellani C, Taylor C, Cuppens H, Dodge J, et al. Cystic fibrosis: terminology and diagnostic algorithms. Thorax 2006;61 (7):627–35.
[14] Knowles M, Gatzy J, Boucher R. Increased bioelectric potential difference across respiratory epithelia in cystic fibrosis. N Engl J Med 1981;305 (25):1489–95.
[15] Knowles MR, Paradiso AM, Boucher RC. In vivo nasal potential difference: techniques and protocols for assessing efficacy of gene transfer in cystic fibrosis. Hum Gene Ther 1995;6(4):445–55.
[16] Wilschanski M, Dupuis A, Ellis L, Jarvi K, Zielenski J, Tullis E, et al. Mutations in the cystic fibrosis transmembrane regulator gene and in vivo transepithelial potentials. Am J Respir Crit Care Med 2006;174 (7):787–94.